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Assessment of Serum Podocalyxin as a Biomarker for Diabetic Nephropathy in Type 2 Diabetes patients in Duhok City

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Abstract

Podocalyxin, a glycosylated cell surface sialomucin of the CD34 family, is a kidney podocyte membrane negatively charged protein and the essential constituent of the glomerular basement membrane charge barrier. Additionally, it has a crucial role in maintaining the glomerular filtration barrier permeability. It has been considered that one of the most important factors in diabetic nephropathy is podocyte injury. In the natural history of diabetic nephropathy and macrovascular complications, podocytes have been shown to be structurally and functionally affected. The current study aimed to determine serum podocalyxin levels in patients with type 2 diabetes mellitus and to analyse its relation to glomerular filtration rate and albuminuria. This cross-sectional study was performed at the diabetic center in Azadi Teaching Hospital and Golan Hospital in Dohuk city from September 2021 to March 2022. Consecutive sampling was applied to select 200 subjects (case group) with T2DM aged 30-65 years and 93 healthy subjects (control group). BMI, eGFR, albuminuria, HbA1c, and serum podocalyxin levels were measured for all study subjects. Serum podocalyxin levels were significantly higher in the case group (*P* =0.019). There was no significant difference in serum podocalyxin levels between case groups (normoalbuminuria, microalbuminuria, and macroalbuminuria). A significant negative correlation between eGFR and serum podocalyxin was found in both control and case group (*P* =0.010 r =-0.267, *P* =0.030 r =-0.154, respectively). Although S.PCX levels had a significant difference between the case and control group and a significant negative correlation with eGFR, it may not be considered as a diabetic nephropathy biomarker.

Keywords: *Podocalyxin, Diabetic Nephropathy, eGFR, Urinary Albumin to Creatinine Ratio (UACR).*

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I. INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is caused by the defective secretion of insulin by pancreatic β-cells, or insulin-sensitive tissues cannot respond to insulin appropriately (Roden and Shulman, 2019). One of the most common long-term diabetes complications is diabetic kidney disease (DKD), often referred to as diabetic nephropathy (DN), about 40% of patients with T2DM are affected. DN is a leading cause of the end-stage renal disease (ESRD) and is defined clinically by elevated urinary albumin excretion or/and the presence of impaired renal function (Barutta *et al*., 2021). Besides increased albuminuria and a decreased estimated glomerular filtration rate (eGFR), the two common indicators of DN (Cole and Florez, 2020), some studies have confirmed the existence of a new biomarker called podocalyxin (Shoji *et al*., 2016; Kostovska *et al*., 2020; Shelbaya *et al*., 2020; Ghorab *et al*., 2020; Xie *et al*., 2021). They measured podocalyxin levels in the urine and confirmed its positive correlation with UACR. Thus, they considered urinary podocalyxin as a biomarker for DN.

Podocalyxin (PCX), a glycosylated cell surface sialomucin of the CD34 family (Le Tran *et al*., 2021), is a kidney podocyte membrane negatively charged protein and the essential constituent of the glomerular basement membrane charge barrier. Additionally, it has a crucial role in maintaining the glomerular filtration barrier permeability (Akankwasa *et al*., 2018). PCX is also normally expressed in a subset of neurons,

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hematopoietic progenitors, mesothelium, and vascular endothelial cells. PCX plays subtle roles in tissue development and remodelling outside the kidney (Le Tran *et al*., 2021).

It has been considered that one of the most important factors in diabetic nephropathy is podocyte injury (Ijpelaar *et al*., 2008). In the natural history of diabetic nephropathy and macrovascular complications, podocytes have been shown to be structurally and functionally affected(Ye *et al*., 2014).

According to the literature, the association of PCX with DN have been confirmed by using a urine specimen (Xie *et al*., 2021). The current study found it of interest to measure PCX levels in serum specimens in order to look if it has the same correlation with DN as urine specimens, through measuring serum PCX levels in patients with type 2 diabetes mellitus and analysing its relation to glomerular filtration rate and albuminuria.

II. Materials And Methods

A. Subjects and study design

This study was designed as a cross-sectional study performed at the diabetic center in Azadi Teaching Hospital and Golan Hospital in Dohuk City from September 2021 to March 2022. Consecutive sampling was applied to select 200 subjects (case group) with T2DM aged 30-65 years and 93 healthy subjects (control group). The participants were selected according to the following inclusion and exclusion criteria: the case group included T2DM subjects and the control group included healthy subjects. The exclusion criteria were renal diseases, chronic diseases (like hypertension, thyroid diseases, and cardiovascular diseases), malignant diseases as well as pregnancy.

Patients who visited the diabetic center at Azadi teaching hospital and Golan hospital during morning working hours (9 am to 11 am) were asked to participate in the research project. Patients who accepted to participate and fulfilled the research criteria were selected as subjects. Consent to participate in the research was signed by each subject. In addition, the following information was collected from each subject; name, age, gender, disease duration, height, and weight.

A blood sample was taken from each subject after verbally verifying that the subject was fasting. A blood sample of 6 mL was drawn from the arm of each subject. 2 mL of blood was collected into the EDTA tube for measuring HbA1c. The other 4 mL were collected into the gel tube and kept for 15 minutes, and then the tube was placed in a centrifuge at 3000 rpm for 10 minutes to separate serum. Half of the serum was kept in the freezer (-20^{o}C) for measuring PCX. The other half was used for the estimation of FBS and creatinine. A fresh urine sample was collected from each subject into a plain tube. The tube was centrifuged at 1000 rpm for 10 minutes. Then a sample of filtered urine was taken to measure albumin and creatinine levels for calculating the urinary albumin to creatinine ratio (UACR).

Random healthy people were invited to participate in the research. People who accepted the invitation were selected as the control group subject. The same data and sample collection method as the case group was followed.

B. Measurements

The biochemistry autoanalyzer, Cobas series 6000, was used to analyze FBS, serum and urine creatinine, urine albumin, and HbA1c. In addition, the Human PCX ELISA Kit (Catalog No: E-EL-H2360, Sensitivity: 0.10 ng/mL, Elabscience Biotechnology Inc. USA) was used to determine the concentration of PCX in serum.

For measuring BMI, this equation was used: BMI = weight (kg)/ height 2 (m^2) (Weir and Jan, 2022). eGFR was calculated using the CKD-EPI equation (The Chronic Kidney Disease Epidemiology Collaboration). To obtain UACR, albuminuria concentration was divided by urinary creatinine concentration [(albuminuria (mg/L) / urinary creatinine (mg/dL)) *100]. UACR was converted to a categorical variable: normoalbuminuria (<30 mg/g), microalbuminuria (30-300 mg/g), and macroalbuminuria(>300 mg/g) (Nah *et al*., 2017).

C. Statistical analysis

The Statistical Package for Social Science (SPSS) version 20 was used to analyze all the data. T-tests and one-way ANOVA were used to compare the groups. The correlation between S.PCX and other parameters was also estimated using Pearson's correlation coefficient. The *P*-value was set at <0.05 to indicate statistical significance.

D. Ethical considerations

The Ethical Committee of the General Directorate of Health in Duhok reviewed and approved the study protocols on 18 August 2021 (Reference number: 18082021-8-24).

III. Results

The comparison of clinical data and laboratory parameters among study groups is summarized in Table 1. The results showed a significant difference between the case and control group regarding all clinical and laboratory data; age *P* <0.001, BMI *P* =0.008, FBS *P* <0.001, HbA1c *P* <0.001, creatinine *P* <0.001, eGFR *P* <0.001, UACR *P* =0.023, S.PCX *P* =0.019.

The comparison between case groups showed no significant differences regarding to S.PCX (*P* =0.584), HbA1c (*P* =0.065), FBS (*P* =0.764), BMI (*P* =0.135), and age (*P*=0.666). Serum creatinine and UACR significantly differed among the three groups (*P* =0.028, *P* <0.001, respectively) (Table 2).

In Table 3, the results revealed a negative correlation for eGFR in case and control groups (*P* =0.030, *P* =0.010, respectively), as shown in scatter plots Figure 1A, 1B, and a positive correlation for serum creatinine in case group (*P* =0.019). However, UACR, FBS, HbA1c, and BMI presented no significant correlation with S.PCX in both groups. In addition, insignificant relation was found between S.PCX and the duration of diabetes.

Table 1. Comparison of clinical data and laboratory parameters among case and control group

Variables	Control Group mean ±SD (n=93)	Case Group mean ±SD (n=200)	*P value*
Gender (male/female)	53/40	98/102	
Age	43.93 ±8.68	50.89 ±8.16	<0.001
BMI (kg/m2)	25.66 ±3.95	27.03 ±4.10	0.008
FBS (mg/dl)	93.04 ±9.97	186.27 ±77.03	<0.001
HbA1c %	5.23 ±0.41	8.79 ±1.93	<0.001
Creatinine (mg/dl)	0.57 ±0.14	0.78 ±0.21	<0.001
eGFR ml/min/1.73m2	120.47 ±10.40	98.59 ±15.32	<0.001
UACR (mg/g)	5.72 ±4.44	62.36 ±237.94	0.023
S.PCX (ng/ml)	118.20 ±41.7	130.60 ±42.07	0.019

Table 2. Comparison of clinical and laboratory data between case groups

Variables	Normo mean ±SD *n* = 154	Micro mean ±SD *n* = 39	Macro mean ±SD *n* = 7	*P value*
Gender (male/female)	82/72	13/26	3/4	
Age	51.05 ±8.08	50.82 ±8.87	48.28 ±6.57	0.687
BMI (kg/m2)	26.82 ±3.76	27.40 ±4.95	29.42 ±5.7	0.135
FBS (mg/dl)	185.30 ±81.23	185.79 ±59.60	210.28 ±73.01	0.705
HbA1c %	8.65 ±1.83	9.21 ±1.90	10.00 ±3.19	0.064
Creatinine (mg/dl)	0.80 ±0.21	0.70 ±0.19	0.86 ±0.28	0.028
eGFR (mL/min/1.73 m²)	97.76 ±14.74	102.89 ±15.79	92.79 ±22.00	0.103
UACR (mg/g)	11.74 ±7.87	91.61 ±63.79	1013.12 ±851.24	<0.001
S.PCX (ng/ml)	129.87 ±41.53	130.58 ±46.61	146.65 ±25.24	0.589

Table 3. Correlation between serum PCX and other laboratory findings in the study groups

Variables	Control group *r* *P value*	Case group *r* *P value*
BMI (kg/m2)	0.101 0.336	0.077 0.276
Duration of diabetes (years)	- -	0.035 0.623
FBS (mg/dl)	-0.001 0.990	0.042 0.552
HbA1c %	0.094 0.371	0.083 0.240
Creatinine (mg/dl)	0.093 0.374	0.166* 0.019
eGFR (mL/min/1.73 m²)	-0.267* 0.010	-0.154* 0.030
UACR (mg/g)	0.033 0.751	0.063 0.374

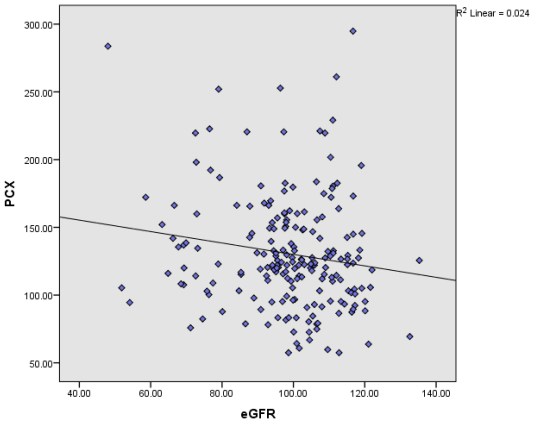

Figure 1A. The correlation between S.PCX and eGFR in the case group.

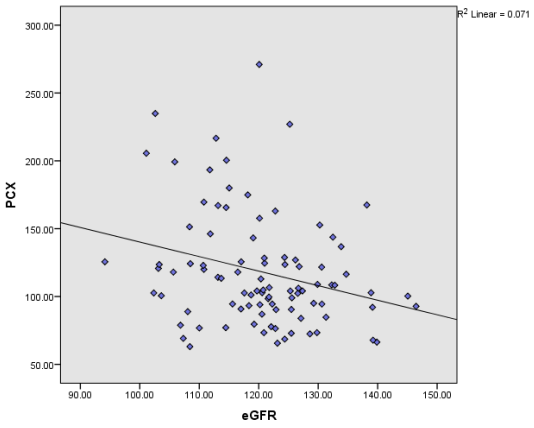

Figure 1B. The correlation between S.PCX and eGFR in the control group.

IV. DISCUSSION

Diabetic nephropathy is a clinical syndrome characterised by albuminuria >300 mg/d or >200 µg/min, confirmed at least two times 3–6 months apart, continuous decline in the eGFR, and high blood pressure (Agarwal, 2009). In general, eGFR and UACR are standard DN markers. Endothelial cells, mesangial cells, and podocytes are three cell types found in the glomerulus that have been found to play crucial roles in DN development (Betsholtz *et al*., 2007). However, it is difficult to determine which cell types are most impacted during DN development without a renal biopsy. It is not appropriate to undertake renal biopsies in all patients with diabetes, particularly in the early stages of DN, as they are an invasive test.

Albuminuria has several perplexing associated issues, including acute illness, cardiac failure, urinary tract infection, and exercise, which reduces its sensitivity and specificity for identifying the early stage of DN (Li *et al*., 2017). Additionally, 30% of T2DM patients may have normoalbuminuria, and not all diabetic patients with microalbuminuria will end up with ESRD.

According to recent research, microalbuminuria is present once a significant renal injury has occurred (Lee and Choi, 2015; Chida *et al*., 2016), and a decline in eGFR is a final outcome of kidney disease (Betsholtz *et al*., 2007). Due to these restrictions, it urgently needs to find more sensitive and specific biomarkers for the identification or early detection of DN. As DN is considered a podocytopathy, measuring specific podocyte proteins, such as PCX, can be used to determine podocyte injury (Al-Rubeaan *et al*., 2017).

An important finding of the present study was that the case group had higher S.PCX levels than the control group. Similar results were found by (Kostovska *et al*., 2020; Shelbaya *et al*., 2020), who reported that urinary podocalyxin (U.PCX) levels were higher in diabetic patients. In addition, higher S.PCX levels were found in patients with macroalbuminuria than in those with normoalbuminuria and microalbuminuria but statistically not significant. The high levels of U.PCX in micro and macroalbuminuria patients were seen by (Ye *et al*., 2014; Ghorab *et al*., 2020) due to the origination of U.PCX from the microvilli or vesicle-like structures in injured podocytes (Hara *et al*., 2012). However, our results showed no correlation between S.PCX and UACR, unlike previous studies conducted on U.PCX and presented a significant positive correlation between them (Ye *et al*., 2014; Shoji *et al*., 2016; Mohamed *et al*., 2016; Kostovska *et al*., 2020; Shelbaya *et al*., 2020; Ghorab *et al*., 2020).

Although we excluded any disease that may affect the results, we could not avoid some diseases that have not yet been diagnosed, such as cardiovascular diseases due to hyperlipidemia. In addition, a study conducted in 2019 showed high S.PCX levels in diabetics with PAD and MI compared to diabetics without PAD and MI (EL-Ashmawy *et al*., 2019). Therefore, the present results of high S.PCX levels in the case group might be due to its expression from various types of cells and tissues, such as neurons, lungs, platelets, and vascular endothelial cells (Doyonnas *et al*., 2005).

The current study showed a negative correlation between S.PCX and eGFR in the case and control group. Similar findings were reported by Mohamed *et al*. (2016), who established a negative correlation between U.PCX and eGFR in patients with T2DM. Conversely, Shoji *et al*., (2016) and Kostovska *et al*., (2020) showed no correlation between the two variables. Serum creatinine and S.PCX were significantly positively correlated in the case group. Similar findings were reported by Kostovska *et al*. (2020) and Shelbaya *et al*. (2020), who reported a positive correlation of U.PCX with serum creatinine. Although there was a correlation between S.PCX and eGFR and the difference in eGFR was significant between patients and healthy controls, their values were within the normal range. In addition, GFR levels can be influenced by obesity, sex, hypertension, glomerular hyperfiltration, and dyslipidemia (Porrini *et al*., 2015). Moreover, serum creatinine levels were within the reference range among diabetic patients. Most diabetics were also normoalbuminuric. However, due to their correlation, S.PCX could be a late marker for DN as serum creatinine.

The main limitation of the present study is the exclusion of diagnosed DN patients. Therefore, further studies are required to confirm this correlation by including DN patients. In the current research, diabetic patients were classified according to UACR following the sampling procedure. Of the 200 patients, macroalbuminuric patients were only seven, microalbuminuria patients were 39, and 154 patients were normoalbuminuric. Accordingly, we cannot rely on these results. Therefore, it was preferable to identify patient groups based on diagnosis, including normoalbuminuria and DN patients, before performing the study.

The outcomes from (Shoji *et al*., 2016) are consistent with the current findings, which show no correlation between U.PCX and HbA1c. Furthermore, BMI in study groups showed no correlation with S.PCX, which was consistent with the findings of a study conducted by (Mohamed *et al*., 2016) that revealed no correlation between BMI and U.PCX.

V. Conclusion

Based on the literature, this is the first study to look for the correlation between S.PCX levels and DN among diabetic patients. S.PCX levels were significantly higher in the case group compared to the control group. There was no correlation between S.PCX and UACR. Because DN patients were not included in this study, S.PCX may not be used as a biomarker for DN. It could be a late marker due to its correlation with serum creatinine, but further research are needed.

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